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Search for hyperhermophilic microorganisms in fluids obtained from the KTB pump test

Harald Huber, Robert Huber, Hans-Dietrich Lüdemann and Karl O. Stetter

Department of Microbiology, University of Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany

Summary. Fluids from the KTB pilot hole were examined for the presence of possibly existing hyperthermophilic microorganisms. Due to the expected low cell concentrations in the fluids, all particles (including organisms) were concentrated about a factor of 10000 by centrifugation. From these samples, enrichments of hyperthermophiles were attempted at incubation temperatures between 85 and 120 °C at low (300 kPa) and high (25000 kPa) pressure. However, no hyperthermophilic microorganisms could be enriched from the concentrated samples.

Key words: Hyperthermophiles - KTB project - Fluids

Introduction

During the last two decades hyperthermophilic microorganisms propagating fastest at temperatures between 80 and 105 °C have been isolated (Stetter et al. 1990). As a rule they do not grow below 60 °C. So far, they have been mainly found in solfataric fields and marine hydrothermal systems in shallow and abyssal depth. Other marine biotopes are active sea mounts. Similar to sea water, submarine volcanic areas contain high concentrations of NaCl and sulfate and exhibit a slightly acidic to alkaline pH (5 - 8.5). Most biotopes of hyperthermophiles are anaerobic or contain only traces of oxygen, due to the low solubility of oxygen at high temperatures and the constant flow of reducing gases like H_2S. Within the hyperthermophiles chemolithotrophic and chemoorganotrophic organisms are known. They gain energy by oxidation of anorganic compounds (e.g. H_2, S^0) or organic material (e.g. cell extracts). Autotrophic species use CO_2 as carbon source and are therefore primary producers of organic material in their biotope. Alternatively, some autotrophs are able to grow heterotrophically on organic substrates. Furthermore, strict organotrophs are known, living by respiration or fermentation of organic matter. So far, there are mainly speculations about the possible existence of hyperthermophiles in fluid inclusions within the lithosphere (Gold 1992). In our project we examined fluid samples from a depth of about 3950 m obtained during the KTB pump test for the existence of microbial life.

Results

Sampling

Due to the expected low concentration of cells within the fluids, we developed a special sampling technique. Particles (and cells) from 2.5 m^3 of degased fluids were concentrated by centrifugation (Padberg Z61, 4°C, flow: 2 l/min). The sediment was resuspended in 250 ml supernatant under a protective CO_2-atmosphere. This suspension was divided into 2 aerobic and 2 anaerobic sam-

ples. Anaerobic samples were obtained by addition of sodium sulfide (final concentration 0.1 %) eliminating dissolved oxygen. In addition 2 l fluid (obtained from the supernatant of the rotor) were stored for enrichment attempts. In total 14 samples were taken by this technique in approximately one week intervals.

At the beginning of the pump test the sediment had an orange colour (samples provided at 8th, 11th, 15th, 28th August, 1st and 3rd September 1991). Due to problems with the pumping equipment in the beginning, this rusty material may have been corrosion products of the pipes. In the samples obtained from October to the end of the pump test in December the amount of sediment decreased significantly (to about 25 % of the original amount) and the colour changed to ochre or blackish.

Enrichment attempts

In the laboratory enrichment attempts were carried out anaerobically and aerobically in synthetic media and in the original water. For some of the enrichments the media were modified in their salt content, according to analyses of the original fluids provided by the field laboratory. The media were inoculated with 1 ml of the original samples and incubated at 85, 100 and 120 °C, respectively. As a control incubations were also carried out at 37 °C.

In order to enrich different groups of hyperthermophiles specific culture conditions and substrates were used:

- Methanogens: strictly anaerobic; H_2/CO_2, methanol, formate, acetate, methylamine;
- Sulfate reducers: strictly anaerobic; sulfate containing media; addition of H_2/CO_2, lactate, formate or cell extracts;
- Sulfur reducers: strictly anaerobic; elemental sulfur or thiosulfate in combination with H_2/CO_2, or organic material;
- Nitrate reducers: nitrate; with and without addition of organic material;
- Sulfide- and sulfur oxidizers: aerobic in acidic media; sulfidic ores, elemental sulfur;
- Possibly existing pyrite formers: strictly anaerobic, addition of Fe^{3+} and H_2S in the presence of CO_2;
- Reducers of oxidic ores: anaeobic; atmosphere H_2/CO_2; haematite; cassiterite, ilmenite;
- Growth on carbohydrates: aerobic and anaerobic; different oils;
- Organotrophic bacteria: sugars, yeast extract, peptone;

In addition, enrichment attempts were carried out in high pressure chambers. A 50 ml glass syringe, filled with 10 ml of the respective culture medium and the specific gas phase, was placed into the steel cylinder. Water was used as hydraulic fluid. The system was pressurized to about 2000 kPa. Hereafter, the cylinder was heated up to 105 °C, resulting in a final pressure of about 30000 kPa. After one to four days the chamber was decompressed and growth was examined by microscopy. Media suitable for the enrichment of methanogens, sulfate reducers, and sulfur reducers were used in these experiments.

Microscopy

The concentrated samples were inspected by phase contrast and by fluorescence microscopy after staining with DAPI (Huber et al. 1985). Straight, curved and strongly bent non-sporeforming rods (length: about 2 - 8 µm) and ovoid cocci (diameter about 1 µm) were present in a concentration of up to 2×10^5 cells/ml.

Enrichments

So far, no cultures of hyperthermophilic microorganismswere obtained from the fluids in spite of hundreds of enrichment attempts. Merely at 37 °C, anaerobic, organotrophic, straight to strongly bent cells were enriched, morphologically similar to Flexistipes sinusarabici, (Fiala et al. 1990). They were unable to grow at temperatures of 50 °C or higher. Most probably they had been contaaminates from the surface and were not further studied in this investigation.

Discussion

The unsuccessful enrichment attempts for hyperthermophiles from the fluids of the KTB pump test may have different reasons:

a) wrong enrichment techniques: This possibility exists, although the screening covered all major groups of hyperthermophiles.

b) in-situ temperatures too high: It cannot be excluded, that the fluids with a temperature of 118 °C are too hot and therefore sterile. So far, no hyperthermophiles are known, growing at temperatures above 110 °C (Stetter et al. 1990).

The existence of microbial life at high temperatures in fluids of the lithosphere is still conceivable but so far unproven. They may exist, but if they are sessile on rocks in the depth, they are not transported or only in very low concentrations. If they were present in the fluids, the concentration of hyperthermophiles must have been less than 1 cell per 10 liter fluid, due to our results. On the other hand, these fluids may be sterile because they have not been infected by organisms which migrated from the surface to the depth by natural cracks.

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References

Fiala G, Woese C R, Langworthy T A, Stetter K O (1990) Flexistipes sinusarabici, a novel genus and species of eubacteria occuring in the Atlantis II Deep brines of the Red Sea. Arch Microbiol 154: 120 - 126

Gold T (1992) The deep, hot biosphere. Proc Natl Acad Sci, USA, 89: 6045 - 6049

Huber H, Huber G, Stetter K O (1985) A modified DAPI fluorescence staining procedure suitable for the visulization of lithotrophic bacteria. System Appl Microbiol 6: 105 - 106

Stetter K O, Fiala G, Huber G, Huber R, Segerer A (1990) Hyperthermophilic microorganisms. FEMS Microbiology Reviews 75: 117 - 124